FORM PTO-1390 (REV. 1-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	41577/252464
	U.S. APPLICATION NO. (If known, see 37 CFR 1.5) unknown 09/744489

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/02317	19 July 1999 (19.07.99)	23 July 1998 (23.07.98)

TITLE OF INVENTION
NUCLEIC ACID DETECTION METHOD BY TRIPLE HELIX FORMATION

APPLICANT(S) FOR DO/EO/US
DREWE, Lisa Joanne; BRIGHTWELL, Gale; HALL, Elizabeth Anne Howlett

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 37 (b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as published (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the published International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36
11. ☐ An Information Disclosure Statement under 37 CFR 1.197 and 1.98
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    a. International Preliminary Examination Report with amended pages 9 and 10 of claims
    b. Certification Under 37 CFR 1.10

I hereby certify that this document is being mailed to Box PCT, Assistant Director for Patents, Washington, D.C. 20231, via "Express Mail Post Office to Addressee" on this 23rd day of January, 2001, Express Mail Label No. EL670008492US

Angela M. Rossi

09/744489

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
unknown	PCT/GB99/02317	41577/252464

17. ☒ The following fees are submitted BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		CALCULATIONS PTO USE ONLY	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1,000.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2) paid to USPTO	$710.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT	=	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		$.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	17	00	X $18.00	$.00	
Independent claims	01	00	X $80.00	$.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$270.00	$.00	
TOTAL OF ABOVE CALCULATIONS			=	$860.00	
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28)			--	$.00	
SUBTOTAL			=	$860.00	
Processing fee of $130.00 for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$.00	
TOTAL NATIONAL FEE			=	$860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40 per property				$40.00	
TOTAL FEES ENCLOSED			=	$900.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ Check in the amount of $900.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 11-0855 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 11-0855.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

Customer Bar Code Label No.

23370
PATENT TRADEMARK OFFICE

SEND ALL CORRESPONDENCE TO:

John S. Pratt, Esq.
KILPATRICK STOCKTON LLP
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309-4530

Dean W. Russell
SIGNATURE

Name: Dean W. Russell
Registration No. 33,452

#6

Attorney Docket: 41577-252464
Patents

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**Drewe et al.**	)
Application No.	**09/744,489**	)
I.A. Filing Date:	**19 JUL 99**	)
Priority Date:	**23 JUL 98**	)
For:	**Nucleic Acid Detection Method by Triple Helix Formation**	)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PCT
Washington, DC 20231

Sir:

Please amend the above-referenced patent application as indicated below. A Response to Notification of Defective Response is enclosed. Also enclosed is a substitute Sequence Listing and a substitute sheet for page 7 of the specification.

In the Specification

Please replace page 7 of the specification with the enclosed substitute sheet.

In the Sequence Listing

Please replace pages 1-3 of the Sequence Listing with pages 1-3 of the enclosed substitute Sequence Listing.

I hereby certify that this correspondence is being deposited with the United States Postal Service addressed to the U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202 on this 20 day of December, 2001.

Jamie L. Greene

Jamie L. Greene - Reg. No. 32,467

Remarks

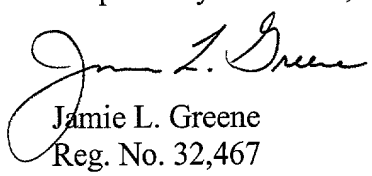
In the enclosed Response to Notification of Defective Response, applicants have amended the Sequence Listing to separate SEQ ID NO 4 into two sequences. Upon entry of this preliminary amendment, the specification will correspond to the sequences set forth in the substitute Sequence Listing to correctly indicate the SEQ ID NOS of these two portions of the peptide nucleic acid below their respective sequences.

On page 7 of the specification, peptide nucleic acid (PNA) 058 was originally identified as a single sequence having the designation SEQ ID NO 4. The right portion of this peptide nucleic acid (PNA) 058 is now labeled as SEQ ID NO 3, and the left portion of originally filed SEQ ID NO 4 remains labeled as SEQ ID NO 4. A marked up copy of page 7 of the specification showing the changes made is enclosed.

Conclusion

Applicants respectfully submit that this Preliminary Amendment places the specification and all claims in the present application in condition for allowance, and such action is courteously solicited. The Examiner is invited and encouraged to contact the undersigned attorney of record at (404) 745-2473 if such contact will facilitate an efficient examination and allowance of the application.

Respectfully submitted,



Jamie L. Greene
Reg. No. 32,467

KILPATRICK STOCKTON LLP
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309-4530
United States of America
Telephone: 404-745-2473
Facsimile: 404-815-6555
Attorney Docket: 41577-252464

strand was designed for Watson-Crick recognition of DNA and the other strand is designed for Hoogsteen recognition of a PNA-DNA duplex and should be optimal for PNA_2DNA triplex stability and thus enhance strand-displacement binding to double-stranded DNA.

PNA058

N TTTTCCCTTCCTTTT LLL TTTTCCTTCCCTTT C [(SEQ ID NO 4)]

(SEQ ID NO 4) (SEQ ID NO 3)

Each PCR product (5μg/ml) was incubated with each PNA probe (10 μg/ml), at 37°C in 0.5 X TE buffer (1 mM Tris.HCl, 0.1 mM EDTA, 5 mM NaCl, pH 8.0) for varying time intervals before the reaction was terminated by adding 150 mM HBS, pH 7.4 on ice. Samples were run on a non-denaturing 12% polyacrylamide gel. The electrophoretic mobility of the triplex PNA_2DNA was compared to the duplex DNA of the relevant PCR product and visualised by EtBr staining. Triplex structures were observed suggesting that PNA can directly detect double-stranded PCR products.

The results of the gel retardation studies showed that single-stranded PNA did not strand invade the PCR products within the first 60 minutes. (This is backed up in the literature where it has been demonstrated that the association of a bis-PNA with a single strand of homopurine DNA gives a complex that is significantly more stable than the one formed with two single PNA strands due to a more favourable entropy of reaction.)

Bis-PNA, however, formed a triplex within the first 10 minutes of reaction.

Example 2

Detection of triplexes on a surface plasmon resonance (SPR) surface.

Biotin labeled bis-PNA (50 μg/ml) was linked to a dextran surface (Biacore, SAchip) via a streptavidin-biotin interaction. A sample of both PCR products (10 μg/ml), in water, was flowed

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PNA058

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Each PCR product (5µg/ml) was incubated with each PNA probe (10 µg/ml), at 37°C in 0.5 X TE buffer (1 mM Tris.HCl, 0.1 mM EDTA, 5 mM NaCl, pH 8.0) for varying time intervals before the reaction was terminated by adding 150 mM HBS, pH 7.4 on ice. Samples were run on a non-denaturing 12% polyacrylamide gel. The electrophoretic mobility of the triplex PNA_2DNA was compared to the duplex DNA of the relevant PCR product and visualised by EtBr staining. Triplex structures were observed suggesting that PNA can directly detect double-stranded PCR products.

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Attorney Docket: 41577-252464
Patents

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**Drewe et al.**	)
Application No.	**09/744,489**	)
I.A. Filing Date:	**19 JUL 99**	)
Priority Date:	**23 JUL 98**	)
For:	**Nucleic Acid Detection Method by Triple Helix Formation**	)

RESPONSE TO NOTIFICATION OF DEFECTIVE RESPONSE

Assistant Commissioner for Patents
Box PCT
Washington, DC 20231

Sir:

In response to the Notification of Defective Response, which was mailed on November 21, 2001, Applicants submit herewith a substitute paper copy of the Sequence Listing and a substitute computer readable form of the Sequence Listing which comprises the nucleotide and amino acid sequences contained in the application as filed. Pursuant to 37 C.F.R. § 1.821(f)

I hereby certify that this correspondence is being deposited with the United States Postal Service addressed to the U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202 on this 20 day of December, 2001.

Jamie L. Greene - Reg. No. 32,467

and 37 C.F.R. § 1.821 (g), the paper copy and the computer readable form are the same and contain no new matter.

Respectfully submitted,

Jamie L. Greene
Reg. No. 32,467

KILPATRICK STOCKTON LLP
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309-4530
United States of America
Telephone: 404-745-2473
Facsimile: 404-815-6555
Attorney Docket: 41577-252464

#3

Patents

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**Drewe et al.**
Application No.	**09/744,489**
Internation Application No:	**PCT/GB99/252464**
I.A. Filing Date:	**19 JUL 99**
Priority Date:	**23 JUL 98**
For:	**Nucleic Acid Detection Method by Triple Helix Formation**

RESPONSE TO NOTIFICATION TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES AND PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

In response to the Notification to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures which was mailed on April 17, 2001, Applicants submit herewith an original paper copy of the Sequence Listing and an original computer readable form of the Sequence Listing which comprises the nucleotide and amino acid sequences contained in the application as filed. No new information has been added. Pursuant to 37 C.F.R. § 1.821(f) and 37 C.F.R. § 1.821 (g), the paper copy and the computer readable form are the same and contain no new matter.

I hereby certify that this correspondence is being deposited with the United States Postal Service addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on this 11 day of June, 2001.

Jamie L. Greene

Jamie L. Greene - Reg. No. 32,467

PRELIMINARY AMENDMENT

Applicants respectfully request that the Sequence Listing, attached hereto on pages numbered 1 - 3, be added to the specification.

Respectfully submitted,

KILPATRICK STOCKTON LLP
2400 Monarch Tower
3424 Peachtree Road, N.E.
(404) 949-3999
Our Docket: 41577-252464

By: Jamie L. Greene
Reg. No: 32,467

SEQUENCE LISTING

<110> Drewe, Lisa

Brightwell, Gale

Howlett, Elizabeth

<120> Nucleic Acid Detection Method by Triple Helix Formation

<130> 41577-252464

<140> US 09/744,489

<141> 2001-01-23

<150> PCT/GB99/02317

<151> 1999-07-19

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IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Applicants: Lisa Joanne DREWE, Gale BRIGHTWELL, And Elizabeth Anne Howlett HALL

International Application No.: PCT/GB99/02317

U.S. Serial No.: unknown

International Filing Date: 19 July 1999 (19.07.99)

U.S. Filing Date: 23 January 2001 (23.01.01)

For: NUCLEIC ACID DETECTION METHOD BY TRIPLE HELIX FORMATION

Box PCT
Assistant Director for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Kindly amend the above-identified patent application prior to examination:

In the Claims

Kindly amend claim 3 at line 1 by deleting "or claim 2."

Kindly amend claim 5 at line 1 by deleting "any one of the preceding claims" and substituting -- claim 1 -- therefor.

Kindly amend claim 6 at line 1 by deleting "any one of the preceding claims" and substituting -- claim 1 -- therefor.

Kindly amend claim 7 at line 1 by deleting "any one of claims 1 to 5" and substituting -- claim 1 -- therefor.

Kindly amend claim 9 at line 1 by deleting "any one of the preceding claims" and substituting -- claim 1 -- therefor.

Kindly amend claim 12 at line 1 by deleting "any one of claims 1 to 8" and substituting -- claim 1 -- therefor.

Express Mail Label No. EL670008492US
U.S.National Phase Entry of PCT/GB99/02317
"Nucleic Acid Detection Method by Triple Helix Formation"
Filed: 23 January 2001
PRELIMINARY AMENDMENT

Kindly amend claim 13 at line 4 by deleting "any one of the preceding claims" and substituting -- claim 1 -- therefor.

Kindly amend claim 14 at lines 1-2 by deleting "any one of the preceding claims" and substituting -- claim 1 -- therefor.

Respectfully submitted,

Dean W. Russell
Reg. No. 33,452

Date: 23 January 2001

KILPATRICK STOCKTON LLP
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309
(404) 815-6528

NUCLEIC ACID DETECTION METHOD BY TRIPLE HELIX FORMATION

The present invention relates to a method of detecting specific target DNA sequences, and in particular to the products of amplification reactions, as well as to reagents and apparatus used in that method.

Many methods are known in order to detect the presence of particular target DNA sequences in a sample. A substantial proportion of these methods require that the DNA is denatured to single stranded form and then this sequence is hybridised or otherwise allowed to bind to a labelled sequence specific probe.

The target sequences are frequently subjected to amplification reactions, for example the polymerase chain reaction or the ligase chain reaction, in order to increase the amount of the target sequence to detectable levels.

Other methods of detecting sequences include the use of intercalating dyes which are incorporated into the sequences during the amplification reaction. However such methods are relatively non specific as the dyes will intercalate with any amplification product, even if they are the result of non-specific amplification products.

Other assays such as the TAQMAN™ assay utilise complex probes which include reporter and quencher moieties during the course of the amplification process. These probes hybridise to single stranded target sequences during the amplification reaction and are then digested by the enzymes carrying out the reaction. The relationship between quencher and reporter molecule of the probe produces a signal which can be monitored. The probes used in this case however, are complex and expensive.

It is known that peptide nucleic acids will strand invade DNA at purine rich sites to form triplex structures (P.E. Nielson et al., Science, 1991, 254, p1497-1506, Turney D.Y. et al,. Proc. Natl. Acad. Sci. USA , 1993, 90, 1667-1670). The mechanism by which this is effected is illustrated diagrammatically hereinafter in Figure 1.

The applicants have found that this phenomenon can be used in detection of target DNA sequences.

A method for detecting the presence of a target nucleic acid sequence in a sample, said method comprising

(a) amplifying said target nucleic acid so that the product of the amplification reaction includes a purine rich region,

(b) contacting the sample with a peptide nucleic acid able to bind to at least a portion of said target sequence; and

(c) detecting the presence of triplex DNA structures.

The method enabling the direct detection of target sequences, for example amplification products without the usual denaturation step required for duplex formation with a nucleic acid probe.

The expression "purine rich region" means that the sequence is suitable for strand invasion by a peptide nucleic acid (PNA). Such regions suitably contain at least four consecutive purine residues.

The reaction in step (a) above is suitably effected in the presence of a buffer, and preferably a low salt buffer for example containing 50mM or less of salt as this favours triplex formation as compared to DNA:DNA duplexes. Furthermore, the pH of the buffer used will depend on the precise nature of the PNA

employed. If C's are used in the PNA strand to strand invade G's on duplex DNA, careful consideration has to be given to the pH of the buffer as the C involved in forming the Hoogsteen base-pair needs to be protonated, requiring a buffer of low pH, for example of less than 4.5.

The peptide nucleic acid used in the method of the invention may be single stranded or it may be bis-PNA. Preferably, the peptide nucleic acid used in the method is a bis-PNA as this results is a faster strand invasion process and a more stable triplex product.

Bis-PNA will comprise of two anti-parallel strands joined by a hydrophilic linker. One strand will be designed for Watson-Crick recognition of DNA within the target sequence, and the other strand is designed for Hoogsteen recognition of a PNA-DNA duplex. Such acids will be optimal for PNA_2DNA triplex stability and thus enhance strand-displacement binding to double-stranded DNA.

Peptide nucleic acids used will suitably contain a sequence of poly-T's or poly-C's.

The target nucleic acid is first subject to an amplification reaction such as the polymerase chain reaction (PCR) or ligase chain reaction (LCR), preferably PCR. The product may be exposed to the peptide nucleic acid during or after the amplification reaction, but is preferably exposed to the peptide nucleic acid after completion of the amplification reaction.

Where the target nucleotide sequence contains or is selected such that it contains a purine rich region, the method can be carried out directly. Where such regions do not exist in the target sequence, they may be introduced during the amplification

reaction. In this case, the amplification will be effected using one or more primers which comprise a plurality of pyrimidines, suitably at the 5' end thereof. This region will chain extend during the extension phase of the amplification (as illustrated in Figure 2 hereinafter). The 3'-end of both amplified strands of the amplification obtained using these primers should now contain the purine rich sites. Indeed, PCR products, that were tagged in this manner, have been cloned and sequenced and were found to have the poly-purine stretches incorporated at their 3'end. This ensures that a suitable PNA binding purine rich region is contained within the amplification product.

Primers of this sort form a further aspect of the invention.

The triplex formed may be detected using various methods in step (b). For example, gel retardation methods may be used. When the product is subjected to gel electrophoresis, for instance on a non-denaturing polyacrylamide gel, and then stained using conventional reagents such as ethidium bromide, the presence of a retarded triplex fraction can be observed.

This method however is relatively slow. Furthermore, comparison with a similar sequence which is not in the form of a triplex is required as a standard.

Preferably therefore, the detection is effected using a capture assay. The capture agent in this case is suitably the PNA sequence which is immobilised on a support. The sample is then contacted with the support whereupon any target sequence present will become associated with the PNA on the surface. It can then be detected using any of the known techniques.

In a particularly preferred embodiment, the support is a waveguide of a detection device which operates using evanescent wave detection. An example of such a device is a surface plasmon resonance detector. This allows the direct and rapid detection of target nucleotide sequence within a sample.

Thus a product of the amplification reaction is simply allowed to flow over the waveguide of such a detector and the presence of an amplicon can be detected in something approaching "real time".

In a further aspect, the invention provides a kit for use in the method of the invention. These kits suitably comprise a PNA designed to form a triplex with a target DNA. Optionally also, it may contain primers which can be used in the amplification of the target DNA, in particular primers which are 5'-tagged with pyrimidines.

The kit may also comprise a waveguide of a evanescent wave detector and particularly a surface plasmon resonance detector having supported thereon, the peptide nucleic acid which specifically binds a target DNA sequence.

The invention will now be particularly described by way of example with reference to the accompanying diagrammatic drawings in which:

Figure 1 illustrates diagrammatically PNA:DNA triplex formation;

Figure 2 illustrates diagrammatically the incorporation of purine rich regions into an amplification product, using 5'-tagging of primers with polyramidine sequences; and

Figure 3 illustrates triplex formation on the surface of a surface plasmon resonance detector.

Example 1

Triplex Formation

The ability of PNA to form triplex structures with PCR products has been demonstrated using gel retardation studies. Two PCR products were chosen for study. One has a sequence capable of forming triplexes with a PNA probe i.e. contains poly-A sites.

PCR82

5'

ATAAATACAACCAACAAAATAAATAGTCATAAAATTGTATACATTAGCAATGCATACC ACAAAGTTCTAAGTACTAAAATAT 3' (SEQ ID NO 1)

The other does not contain poly-A sites and acts as a negative control.

PCR 175

5'

GCGAAACGGAACATAGCCCAAACCAAGAGGCTTGCCTCTTGGGGTTGTAGGACATTCT ATACGGAGTTACAAAGGAAGCAGGTAGACGAAGCGACCTGGAAAGGTCCGTCGTAGAGGGTAAC AACCCCGTAGTCGAAACTTCGTTCTCTCTTGAATGTATCCTGAGTACGGCGGAACACGTGAAA 3' (SEQ ID NO 2)

Two types of PNA probe were used, one was a linear sequence and contains a sequence of poly-T's

PNA057

N TTTTCCTTCCCTTTT C (SEQ ID NO 3)

The other, a bis-PNA of the same linear sequence but composed of two anti-parallel strands joined by a hydrophilic linker. One

strand was designed for Watson-Crick recognition of DNA and the other strand is designed for Hoogsteen recognition of a PNA-DNA duplex and should be optimal for PNA_2DNA triplex stability and thus enhance strand-displacement binding to double-stranded DNA.

PNA058

N TTTTCCCTTCCTTTT LLL TTTTCCTTCCCTTTT C (SEQ ID NO 4)

Each PCR product (5 µg/ml) was incubated with each PNA probe (10 µg/ml), at 37°C in 0.5 X TE buffer (1 mM Tris.HCl, 0.1 mM EDTA, 5 mM NaCl, pH 8.0) for varying time intervals before the reaction was terminated by adding 150 mM HBS, pH 7.4 on ice. Samples were run on a non-denaturing 12% polyacrylamide gel. The electrophoretic mobility of the triplex PNA_2DNA was compared to the duplex DNA of the relevant PCR product and visualised by EtBr staining. Triplex structures were observed suggesting that PNA can directly detect double-stranded PCR products.

The results of the gel retardation studies showed that single-stranded PNA did not strand invade the PCR products within the first 60 minutes. (This is backed up in the literature where it has been demonstrated that the association of a bis-PNA with a single strand of homopurine DNA gives a complex that is significantly more stable than the one formed with two single PNA strands due to a more favourable entropy of reaction.)

Bis-PNA, however, formed a triplex within the first 10 minutes of reaction.

Example 2

<u>Detection of triplexes on a surface plasmon resonance (SPR) surface.</u>

Biotin labelled bis-PNA (50 µg/ml) was linked to a dextran surface (Biacore, SAchip) via a streptavidin-biotin interaction. A sample of both PCR products (10 µg/ml), in water, was flowed

over this sensor surface and were detected by a change in refractive index. The SPR system could differentiate between purine-rich and non-purine rich PCR products in near real time (See Figure 3).

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Claims

1. A method for detecting the presence of a target nucleic acid sequence in a sample, said method comprising

(a) amplifying said target nucleic acid so that the product of the amplification reaction includes a purine rich region;

(b) contacting the sample with a peptide nucleic acid able to bind at least a portion of said target sequence; and

(c) detecting the presence of triplex structures.

2. A method according to claim 1 wherein the peptide nucleic acid is bis-PNA.

3. A method according to claim 1 or claim 2 wherein the amplification product is exposed to the peptide nucleic acid during or after the amplification reaction.

4. A method according to claim 3 wherein the amplification product is exposed to the peptide nucleic acid after completion of the amplification reaction.

5. A method according to any one of the preceding claims wherein the amplification reaction is a polymerase chain reaction.

6. A method according to any one of the preceding claims wherein the target nucleic acid contains a purine rich region.

7. A method according to any one of claims 1 to 5 wherein a purine rich region is introduced into the amplification product during the amplification reaction.

8. A method according to claim 7 wherein primers used in the amplification comprise a plurality of pyrimidines at the 5' end thereof.

9. A method according to any one of the preceding claims wherein the peptide nucleic acid is immobilised on a support.

10. A method according to claim 9 wherein the support is a waveguide of a detection device.

11. A method according to claim 10 wherein the detection device is a surface plasmon resonance detector.

12. A method according to any one of claims 1 to 8 wherein the triplex structure is detected by a gel retardation method.

13. The use of a primer comprising a sequence which hybridises to an end region of a target nucleic acid sequence, and a plurality of pyrimidine residues at a 5' region thereof; in a method according to any one of the preceding claims.

14. A kit for carrying out a method according to any one of the preceding claims, said kit comprising a peptide nucleic acid sequence which is specific for a target nucleotide sequence, and a primer comprising a sequence which hybridises to an end region of a target nucleic acid sequence, and a plurality of pyrimidine residues at a 5' region thereof.

15. A kit according to claim 14 wherein the peptide nucleic acid is immobilised on a waveguide of an evanescent wave detector apparatus.

16. A kit according to claim 15 wherein the evanescent wave detector apparatus is a surface plasmon resonance detector.

17. A method for detecting a nucleotide sequence according to claim 1, substantially as hereinbefore described.

Fig.1.

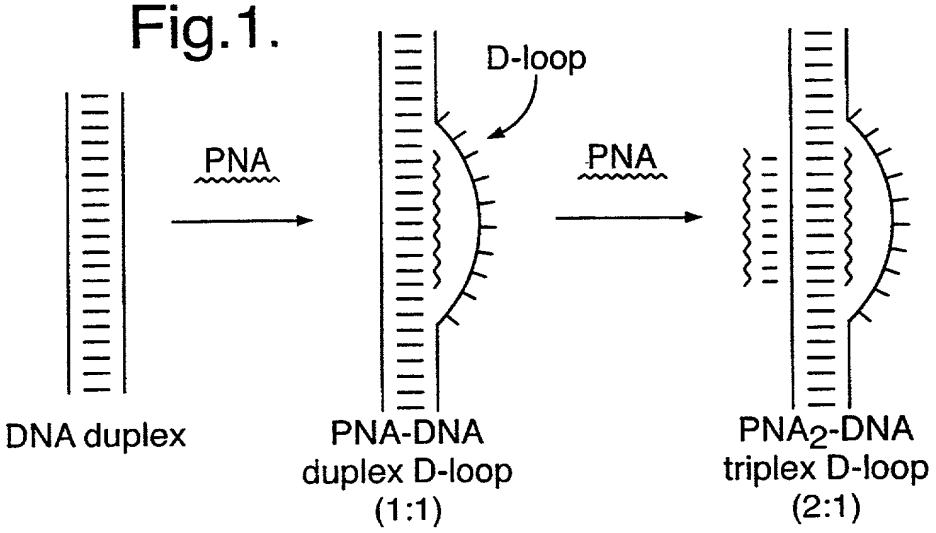
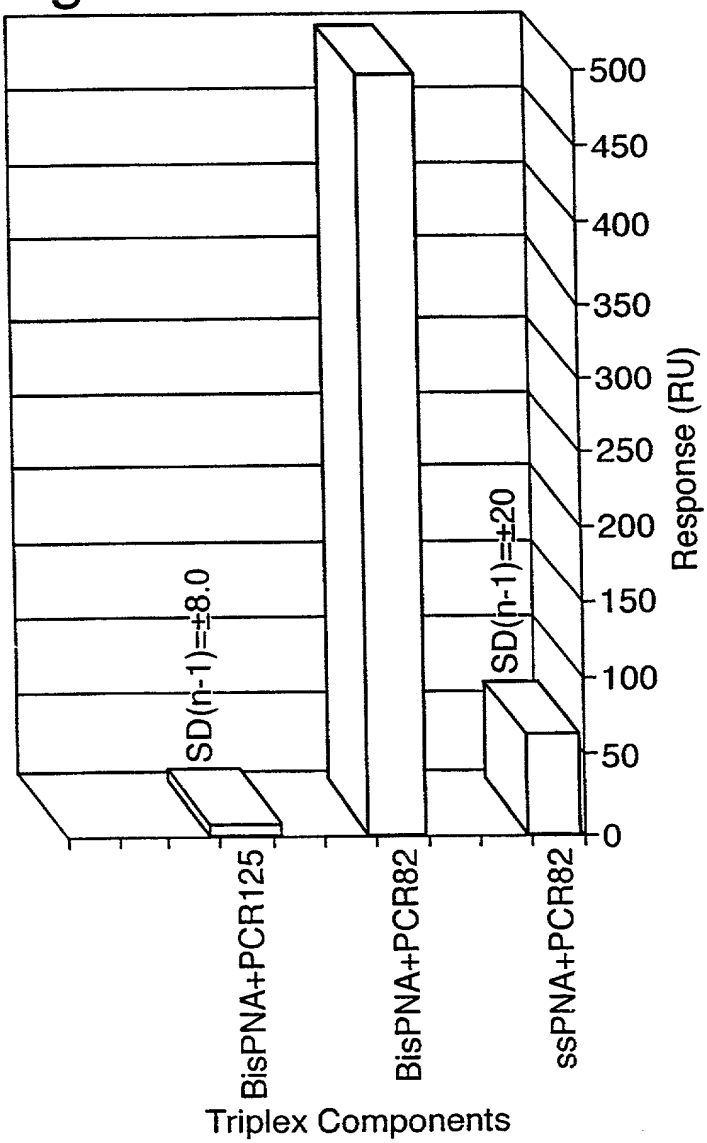

D-loop
PNA
PNA
DNA duplex
PNA-DNA
duplex D-loop
(1:1)
PNA2-DNA
triplex D-loop
(2:1)

Fig.3.


500
450
400
350
300
250
200
150
100
50
0
Response (RU)
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SD(n-1)=±20
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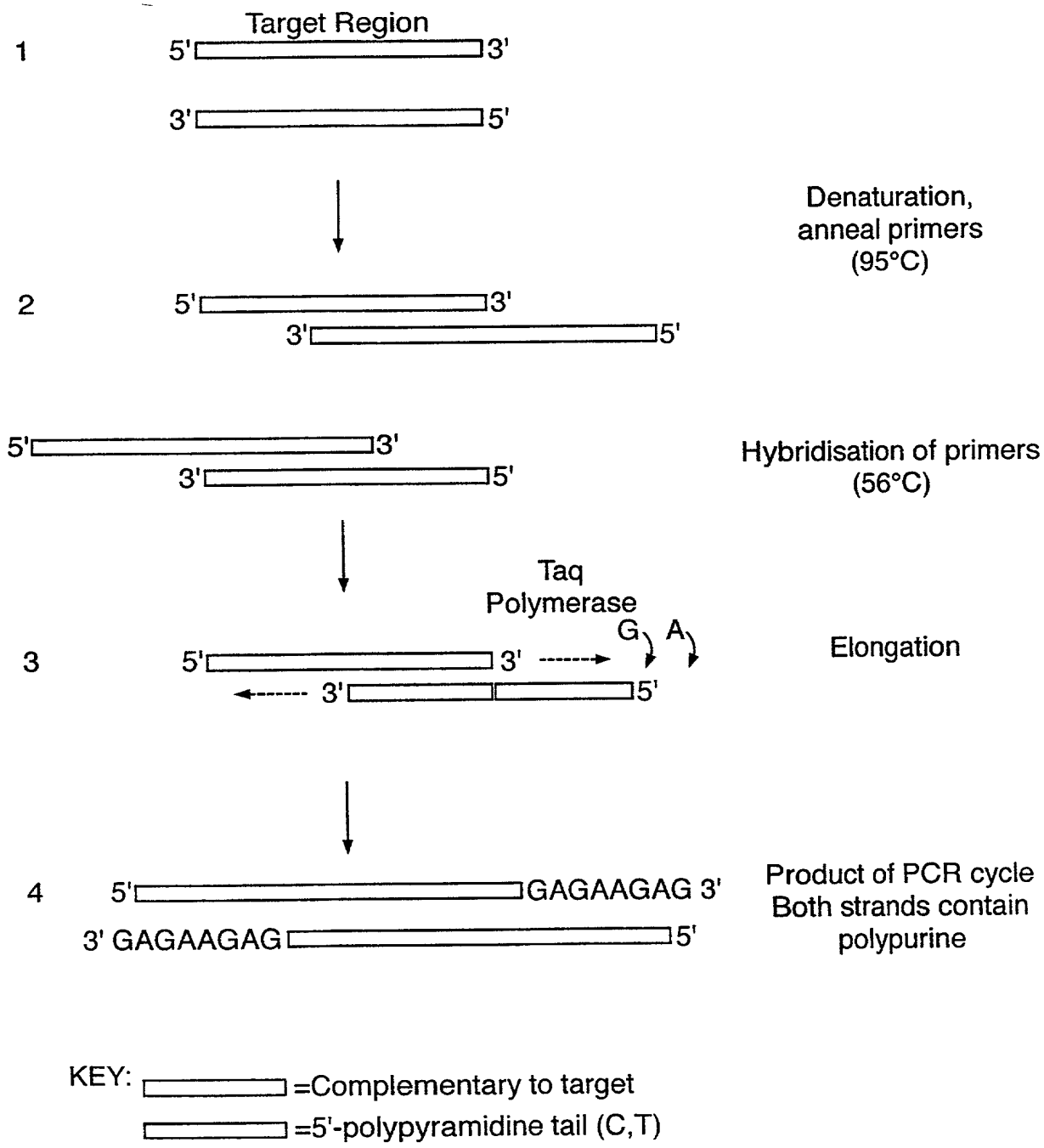
BisPNA+PCR82
ssPNA+PCR82
Triplex Components

Fig.2.


Target Region
1
5'
3'
3'
5'
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2
5'
3'
3'
5'
5'
3'
3'
5'
Hybridisation of primers (56°C)
Taq Polymerase
G
A
3
5'
3'
3'
5'
Elongation
4
5'
GAGAAGAG 3'
3' GAGAAGAG
5'
Product of PCR cycle Both strands contain polypurine
KEY:
=Complementary to target
=5'-polypyramidine tail (C,T)

Attorney Docket No. _____

DECLARATION FOR PATENT APPLICATION

☒ Original ☐ Supplemental ☐ Substitute ☐ PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below), or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NUCLEIC ACID DETECTION METHOD BY TRIPLE HELIX FORMATION

(Title of the Invention)

the specification of which (check one)

☐ is attached hereto

☐ was filed on _____ as U. S. Application Serial Number _____

☒ was filed as PCT International Application Number PCT/GB99/02317 on 19 July1999

and was amended under PCT Article 19 on

4 July 2000

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 (a) - (d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified, by checking the box below, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Applications			Priority Claimed		Copy Attached	
Application Number	Country	Foreign Filing Date	YES	NO	YES	NO
9815933.8	GB	23 July 1998	X			

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below and claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT international application(s) designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Parent Application Number	Filing Date	Status (Mark Appropriate Column Below)		
		Patented	Pending	Abandoned
PCT/GB99/02317	19 July 1999		X	

As a named inventor, I hereby revoke all prior powers and appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

KILPATRICK STOCKTON LLP, Suite 2800, 1100 Peachtree Street, Atlanta, Georgia 30309-4530

Attorney and/or Agent	Registration No.
Charles Y. Lackey	22,707
John M. Harrington	25,592
John S. Pratt	29,476
A. Jose Cortina	29,733
James L. Ewing, IV	30,630
Charles W. Calkins	31,814
George T. Marcou	33,014
Bernard J. Graves, Jr.	33,239
Dean W. Russell	33,452
Richard T. Peterson	35,320
Charles T. Simmons	35,359
Nora M. Tocups	35,717
Bruce D. Gray	35,799
Theodore R. Harper	35,890
Geoff L. Sutcliffe	36,348
Stephen B. Parker	36,631
Pat Winston Kennedy	36,970
Mitchell G. Stockwell	39,389
Michael J. Turton	40,852
Yoncha L. Kundupoglu	41,130

Attorney and/or Agent	Registration No.
Benjamin D. Driscoll	41,571
Alana G. Kriegsman	41,747
J. Steven Gardner	41,772
James J. Bindseil	42,326
Camilla Camp Williams	43,992
Carl B. Massey	44,224
R. Whitney Winston	44,432
John William Ball, Jr.	44,433
Dawn-Marie Bey	44,442
Tiep H. Nguyen	44,465
Michael J. Dimino	44,657
Kristin L. Johnson	44,807
J. Jason Link	44,874
Bambi F. Walters	45,197
J. Michael Boggs	P46,563
Adam E. Crall	P46,646
Kyle M. Globerman	P46,730
Tywanda L. Harris	P46,758
Kristin D. Mallatt	P46,895
Cynthia B. Rothschild	P47,040

I acknowledge the above-listed attorneys and agents and their firm Kilpatrick Stockton LLP represent my employer (if I am an employee and this application has been or will be assigned to my employer) or the entity with which I have contracted (if I am an

independent contractor and this application has been or will be assigned to such entity) and in such cases do not represent me individually. I further acknowledge I have not established, nor will I seek to establish, any personal attorney/client relationship with Kilpatrick Stockton LLP in connection with this application and understand that, should I require legal representation, I will obtain such, at my expense, other than through Kilpatrick Stockton LLP.

Send Correspondence to: John S. Pratt, Esq.
Kilpatrick Stockton LLP
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309-4530

Customer No. 23373

Direct telephone calls to: Dean W. Russell, Esq. (404) 815-6528

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 Full name of first inventor Lisa Joanne DREWE
Inventor's signature [signature] Date 7th October 2000
Residence Salisbury GBN
Citizenship GB
Post Office Address CBD Porton Down, Salisbury, Wiltshire, SP4 0JQ. GB.

2-00 Full name of second inventor Gale BRIGHTWELL
Inventor's signature [signature] Date 18-10-2000
Residence Salisbury
Citizenship GB
Post Office Address CBD Porton Down, Salisbury, Wiltshire, SP4 0JQ. GB.

3-00 Full name of first inventor Elizabeth Ann Howlett HALL
Inventor's signature Elizabeth Hall Date 19 Sept 2000
Residence Salisbury
Citizenship GB
Post Office Address CBD Porton Down, Salisbury, Wiltshire, SP4 0JQ. GB.

SEQUENCE LISTING

<110> Drewe, Lisa J.

Brightwell, Gale

Howlett, Elizabeth A.

<120> Nucleic Acid Detection Method by Triple Helix Formation

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<141> 2001-01-23

<150> PCT/GB99/02317

<151> 1999-07-19

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